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(54) Particles and Procedures for the Determination of Antigens and/or Antibodies Using the Particles

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- Abstract -

The invention concerns particles and a procedure for the simultaneous determination of several antigens or antibodies in a liquid using these particles. The mixture of particles contains populations of particles, which differ from each other in the following way:
Each population exhibits a specific combination of the following characteristics: 1) fluorescent substances with differing emission spectra, 2) quantity of these fluorescent substances, 3) particle size. Each particle population is loaded furthermore with a different type of antibody or antigen. Using this mixture of particles, the simultaneous investigation of several types of antigen or antibody can be carried out a time and effort saving manner. The particle mixture is mixed with the liquid containing the antibodies or antigens to be determined. The subsequent reaction steps correspond to the steps of a conventional immunofluorescence procedure. Finally, each particle is measured using a measuring device (e.g. flow cytometer) for its fluorescence (emission spectrum and intensity) and size. One the basis of the measured data, a computer identifies the particle and correlates the measured immunofluorescence with a defined specificity.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A test agent with labelling for analyzing a plurality of different antigens and/or antibodies in a liquid sample, characterized by neutral carrier particles which are labelled individually or in groups with a fluorescent labelling substance and which are loadable with antibodies or antigens differing from one another for determining antigens and/or antibodies.

2. A test agent according to claim 1, characterized in that said particles are approximately spherical and of approximately the same size.

3. A test agent according to claim 2, characterized in that said particles are of a size in the range of approximately 1 μm to approximately 100 μm.

4. A test agent according to claim 1, characterized in that said particles are applied in several discrete sizes, whereby they can be instrumentally categorized with great certainty, according to their size, into particle populations which can be distinguished one from another, whereby the size of the particles serves as an additional labelling (distinguishing) characteristic.

5. A test agent according to claim 1, characterized in that said particles consist of a material selected from the group consisting of plastic, rubber, polysaccharide polymer (e.g. agar) or another polymer or glass or that said particles are cells, e.g. erythrocytes.

6. A test agent according to claim 2, characterized in that said particles consist of a material selected from the group consisting of plastic, rubber, polysaccharide polymer (e.g. agar) or another polymer or glass or that said particles are cells, e.g. erythrocytes.

7. A test agent according to claim 3, characterized in that said particles consist of a material selected from the group consisting of plastic, rubber, polysaccharide polymer (e.g. agar) or another polymer or glass or that said particles are cells, e.g. erythrocytes.

8. A test agent according to claim 4, characterized in that said particles consist of a material selected from the group consisting of plastic, rubber, polysaccharide polymer (e.g. agar) or another polymer or glass or that said particles are cells, e.g. erythrocytes.

9. A test agent according to claim 1, 2 or 3, characterized in that said particles are loaded by direct binding of the antigen to the particle or characterized in that the particles are initially loaded with the specific antibody and then the antigen is bound to this antibody by immunological reaction (particle-antibody-antigen).

10. A test agent according to claim 4, 5 or 6, characterized in that said particles are loaded by direct binding of the antigen to the particle or characterized in that the particles are initially loaded with the specific antibody and then the antigen is bound to this antibody by immunological reaction (particle-antibody-antigen).

11. A test agent according to claim 7 or 8, characterized in that said particles are loaded by direct binding of the antigen to the particle or characterized in that the particles are initially loaded with the specific antibody and then the antigen is bound to this antibody by immunological reaction (particle-antibody-antigen).

12. A process for the determination of antigens or antibodies using particles variously labelled by labelling substances selected from the group of fluorescing substances, colouring agents or pigments and/or their size, characterized in that said particles are loaded with antigens or antibodies differing from one another, that a mixture of such loaded particles is mixed with a liquid containing the antibodies or antigens to be investigated or determined, and that, after a reaction period in which the antibodies or antigens to be determined are bound to the antibodies or antigens fixed to the particles, with which the particles are loaded, the antibodies or antigens are identified by subsequent reaction steps and measurements.

13. A process according to claim 12, wherein said labelling substances exhibit different but defined emission spectra.

14. A process according to claim 13, wherein said labelling substance is one or more fluorescing substances with predetermined differing concentrations.

15. A process according to claim 14, wherein said labelling substances are positioned on the surface of said particles, embedded within said particles or bound to the molecules of the material of said particles.

16. A process according to claim 15, wherein said particles are approximately spherical and of approximately the same size.

17. A process according to claim 16, wherein the size of said particles is in the range of approximately 1 μm to approximately 100 μm.

18. A process according to claim 17, wherein said particles are applied in several discrete sizes, whereby they can be instrumentally categorized with great certainty, according to their size, into particle populations which can be distinguished one from another, whereby the size of the particle served as an additional labelling (distinguishing) characteristic.

19. A process according to claim 18, wherein said particles consist of a material suitable for loading with antigen or antibody.

20. A process according to claim 19, wherein said material is plastic, rubber, polysaccharide polymer (e.g. agar) or another polymer or glass or wherein said particles are cells, e.g. erythrocytes.

21. A process according to claim 20, wherein the said surfaces of said particles are loaded respectively with different antigens or antibodies.

22. A process according to claim 21, wherein said loading of said particles with said antigen is either carried out by direct binding of the antigen to the particle or such that said particles are initially loaded with the specific antibody and then said antigen is bound to said antibody by immunological reaction (particle-antibody-antigen).

3. A process according to claim 19, characterized in that the following procedural steps are carried out for the identification of the bound antibodies:

(a) washing of the particles after completion of the reaction time, for the removal of non-bound substances;

(b) in the case of the investigation of antibodies, addition of a liquid with labelled antibodies, which react species-specifically with the antibodies to be determined or investigated (e.g. anti-human globulin in the investigation of human material);

(c) washing for the removal of non-bound substances; and

(d) analysis of the labelling of the individual particles.

24. A process according to claim 23, wherein fluorescence-labelled antigens are also employed for the determination of antibodies.

25. A process according to claim 19, characterized such that, for the identification of the bound antigens, and before the addition of the liquid with labelled antibodies, a liquid containing a mixture of non-labelled antibodies is added, which reacts specifically with the antigens to be determined, followed by a washing step after the completion of a preset reaction time, and that the labelled antibodies which are used react species specifically with the non-labelled antibodies which are used.

26. A process according to claim 25, wherein fluorescence-labelled antigens are also employed for the determination of antibodies.

27. A process according to claim 24, characterized in that the antibodies or antigens are labelled with fluorescent substances.

A process according to claim 26, characterized in that the antibodies or antigens are labelled with fluorescent substances.

29. A process according to claim 27, characterized in that the fluorescein-labelled antibodies or antigens are added simultaneously with the liquid to be investigated to the particles, or after a certain reaction time following addition of the liquid to be investigated, without prior washing of the particles.

30. A process according to claim 28, characterized in that the fluorescein-labelled antibodies or antigens are added simultaneously with the liquid to be investigated to the particles, or after a certain reaction time following addition of the liquid to be investigated, without prior washing of the particles.

31. A process according to claim 29, characterized in that the washing of the particles after the reaction time with the liquid to be investigated and the fluorescein-labelled antibodies is deleted.

32. A process according to claim 30, characterized in that the washing of the particles after the reaction time with the liquid to be investigated and the fluorescein-labelled antibodies is deleted.

33. A process according to claim 31, characterized in that the analysis is carried out using fluorescence photometry or photometry such that the fluorescence data of each particle are determined (identification fluorescence of the particles and fluorescence of the labelled antibodies) and evaluated.

1. A process according to claim 32, characterized in that the analysis is carried out using fluorescence photometry or photometry such that the fluorescence data of each particle are determined (identification fluorescence of the particles and fluorescence of the labelled antibodies) and evaluated.

35. A process according to claim 33, characterized in that the emission spectrum is excited by radiation with a broad spectrum.

36. A process according to claim 33, characterized in that a certain line of the emission spectrum is excited by radiation of a defined wavelength, or that several discrete lines are excited by radiation with several defined wavelengths.

37. A process according to claim 34, characterized in that the emission spectrum is excited by radiation with a broad spectrum.

38. A process according to claim 34, characterized in that a certain line of the emission spectrum is excited by radiation of a defined wavelength, or that several discrete lines are excited by radiation with several defined wavelengths.

39. A process according to claim 12, characterized in that, for the identification of the particles, the fluorescence spectrum or spectra are measured and evaluated on the basis of wavelength and/or intensity of the fluorescence radiation of the labelling substance or substances and/or the particle size or that the labels of colouring agents or pigments are evaluated on the basis of the reflected light.

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). A process according to claim 18, characterized in that, for the identification of the particles, the fluorescence spectrum or spectra are measured and evaluated on the basis of wavelength and/or intensity of the fluorescence radiation of the labelling substance or substances and/or the particle size or that the labels of colouring agents or pigments are evaluated on the basis of the reflected light.

41. A process according to claim 33 or 39, characterized in that the evaluation is carried out on the basis of the categorization of the immunological reactions which have taken place in one or several antigen/antibody systems.

42. A process according to claim 34 or 40, characterized in that the evaluation is carried out on the basis of the categorization of the immunological reactions which have taken place in one or several antigen/antibody systems.

43. A process according to claim 31, characterized in that the particles are directed through a thin tube in the form of a dispersion (sol or suspension) following the procedural steps in claim 31 in a concentration which permits individual determination, and are measured (flow-cytometer).

44. A process according to claim 32, characterized in that the particles are directed through a thin tube in the form of a dispersion (sol or suspension) following the procedural steps in claim 32 in a concentration which permits individual determinations, and are measured (flow-cytometer).

45. A process according to claim 12, characterized in that a mixture of the labelled and loaded particles is fixed to a carrier.

46. A process according to claim 45, characterized in that said carrier is of plastic or glass.

47. A process according to claim 13 or 45, characterized in that the liquid is added after fixing of the particles.

Particles and procedures for the determination of antigens and/or antibodies using the particles

The invention concerns a particle and a procedure for the determination of antigens and/or antibodies using these particles.

There are various procedures for the determination of antigens or antibodies, based on the principles of agglugination, precipitation, complement binding reaction, immunofluorescence, radioimmunoreaction, enzyme immunoreaction etc. All of these procedures have in common, that only one type of antigen or antibody can be determined at a time.

However, it is commonly the case that a search is for several types of antigen or antibody, e.g. for the differential diagnosis of infectious or other diseases, in screening tests in healthy subjects, e.g. in serological diagnosis of tumors (multiple tumor antigens), or in allergy tests (several allergens) or in the determination of the immunological status of the organism. According to this state-of-the-art of the techniques, particular investigations have to be performed for each type of antigen or antibody which is sought. This represents a multiple investment of time and material.

The object underlying the discovery is to reduce the expenditure of time and material in the investigation and determination of several types of antigen or antibody.

According to the present invention, the solution of this problem is possible using a mixture of particles characterized by one or more distinguishable labeling substances. Thus the particles in the mixture can be distinguished on the basis of their different labels. Also on the basis of this fact, the fluorimetrically measured immunoreaction between the antigen or antibody with the particle, loaded with antigens or antibodies, and the antibody or antigen in the liquid to be investigated, can be categorized into a particular specificity, which represents the combination of labeling signals of the particles.

Accordingly, in the present invention, there is provided a test agent with labelling for analyzing a plurality of different antigens and/or antibodies in a liquid sample, characterized by neutral carrier particles which are labelled individually or in groups with a fluorescent labelling substance and which are loadable with different antibodies or antigens for determining antigens and/or antibodies.

A further aspect of the present invention is a procedure for the determination of antigens or antibodies using particles variously labeled by labeling substances selected from the group of fluorescing substances, colouring agents or pigments and/or their size, characterized in that said particles are loaded with different antigens or antibodies, that a mixture of such loaded particles is mixed with a liquid containing the antibodies or antigens to be investigated or determined, and that, after a reaction period in which the antibodies or antigens to be determined are bound to the antibodies or antigens fixed to the particles, with which the particles are loaded, the antibodies or antigens are identified by subsequent reaction steps and measurements. Using this procedure, it is possible, through the categorization of the reacting antigens or antibodies towards the distinguishable particles of the mixture, to determine the antigens or antibodies by the measurement of immunofluorescence and by probing and analysis of the labels.

The invention is described below in more detail using the following example, whereby reference is made to the accompanying drawings which illustrate embodiments of the invention as follows:

Figure 1 depicts a particle adapted to the determination of bound antibodies according to the present invention; and
Figure 2 depicts a particle adapted to the determination of bound antigens according to the present invention.

A serum is to be investigated for antibody types 2 and 16, e.g. Ak_1, Ak_2, ..., Ak_j, ..., Ak_n. Groups (populations of small particles 4, preferably in the form

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of spheres with a diameter of approximately 10 µm and of a suitable carrier material (plastic or polysaccharide polymer, e.g. agar), are loaded each with a type of antigen, 6 or 12, Ag_1, Ag_2,..., Ag_j,..., Ag_n. Each type of antigen ist bound to a particular type of particle, which were previously labeled in the following way.

The particles 4 are labeled with a combination of substances 8, whose fluorescence spectrum is defined and can be determined fluorimetrically. The labeling can also be carried out in a simple manner, such that the individual particle populations are only labeled with one fluorescing labeling substance with a particular concentration, differing however from particle population to particle population, or with several labeling substances with a particular concentration, differing however from particle population to particle population. This type of labeling (labeling with distinguishable concentrations of the same fluorescing substance) can be combined with labeling with labeling using a combination of fluorescing substances with differing emission spectra. The evaluation is then performed on the basis of the spectrum and/or intensity of the emitted fluorescence. In this way, using one labeling substance, applied in two distinguishable concentrations (0% and 100% of a particular concentration), $2^1 = 2$ particle populations can be distinguished (particles with the substance and particles without the substance). If during the labeling, the substance is applied in three distinguishable concentrations, e.g. 0%, 50% and 100% of a given concentration, then $3^1 = 3$ particle populations can be distinguished. With n labeling substances with differing emission spectra, each applied in m^n distinguishable concentration, the number of labeling possibilities amounts to m , e.g. with three labeling substances each in ten distinguishable concentrations, $10^3 = 1000$ different particle populations can be characterized and identified. The labeling of the particles occurs

during or after their production. For each type of antigen (Ag_j), a particle population (P_j) definable fluorescence spectrophotometrically or by its size is applied. The antigen or antibody is chemically or physically bound to the particle. This is done separately for each type of antigen or antibody. Thereafter, all the particles are mixed in the desired combination. A mixture of particles P_1, P_2, ..., P_j, ..., P_n, loaded with the respective antigens Ag_1, Ag_2, ..., Ag_j, ..., Ag_n is thus produced.

This mixture of particles is mixed with the liquid (e.g. blood serum), containing the antibodies to be determined. After a reaction time, the the antibodies to be determined, 16 and 2, bind specifically to the corresponding antigens 12 and 6. After a washing step for the particles using a washing fluid for the removal of non-bound substances, the particles are mixed with a solution of fluorescein-labeled antibodies 10, which react species specifically with the antibodies to be determined. These fluorescein-labeled antibodies react with all antibodies (every antigen specificity) of the animal species, from which the antibodies to be determined originate. After the reaction time, in which the antibodies 10 are bound to the antibodies 16 and 2, the particles are again washed to remove non-bound fluorescein-labeled antibodies. Then, not only the fluorescence of each individual particle-bound antibody 10 (immunofluorescence as a parameter for the immune reaction which has taken place) is measured, but also the fluorescence label which identifies the particle, the size of the particle using a suitable measuring device, are also measured. A flow-cytometer which measures the fluorescence data (and also the size) of each individual particle is suitable for this. The data are processed by a computer, whereby

the immunofluorescence is appointed to the appropriate particle population. In this way, a profile of the various antigen-antibody reactions $Ag_1 Ak_1$,...,$Ag_n Ak_n$ is presented.

The procedure described above is equally applicable to the determination of antigens 12 in the liquid to be investigated. In this case, after the first reaction and washing step, a mixture of antibodies 16 towards all the antigens to be investigated is added. These antibodies originate preferably from another animal species than antibodies 14. After renewed reaction and washing step, the fluorescein-labeled antibodies 10, which react species specifically with the antibodies 16, are added. The measurement follows as in the investigations for antibodies.

The procedure can also be carried out in the following way, which deviates from the flow-cytometry:

The particle mixture is fixed to a slide, e.g. to the base of a microtitre plate. The serum to be investigated is then applied to this slide (particle mosaic). After a reaction time, the antibodies present in the serum bind to the corresponding antigens on the particles. The non-bound substances are removed by subsequent washing.

In a second step, a fluorescence-labeled globulin antibody 10 is applied, which is specific for the animal species from which the antibodies to be investigated 2 or 16 originate. In this second reaction, the fluorescence-labeled globulin antibody 10 binds to the antibodies (globulins) 2 and 16, which were bound to the particles 4 in the first reaction step. The non-bound material is removed in a further washing step.

The preparation is then investigated photometrically using a fluorescence photomicroscope, which is fitted out with filters etc, so that it can measure the spectrum and intensity of the fluorescence radiation emitted by a single particle. For the investigation, it is expedient to guide the detector of a fluorescence microscope in a predetermined course, e.g. columnwise, over the particle mixture on the slide which is to be investigated. This can also be performed automatically with a suitable appliance, where required.

The data are evaluated using a computer. The investigated particle is indentified on the basis of the fluorescence spctrum of the labels contained in the particle, e.g. as particle P_j. Thus, the measured fluorescence, which derives from the globulin antibody (immunofluorescence) can be attributed to the immunological reaction $Ag_j\,Ak_j$. In this way, the emission data of a large number of particles can be gathered one after the other automatically, and evaluated by the computer. The data of all the particle populations can be assimilated and processed according to the statistical distribution of the particles. Thus, a profile of the different antigen-antibody reactions $Ag_1\,Ak_1$, $Ag_2\,Ak_2$,...,$Ag_n\,Ak_n$ can be presented.

The emission spectrum or the emission spectra of the labels of the particles can be excited by a radiation of a wide spectrum; however, a defined line of the emission spectrum can also be excited by radiation of a defined wavelength or several defined lines through excitation by several defined wavelngths.

FIG.1

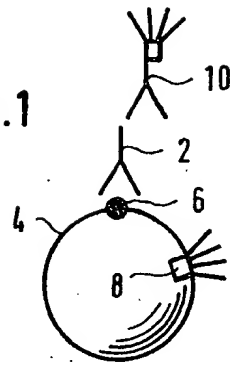
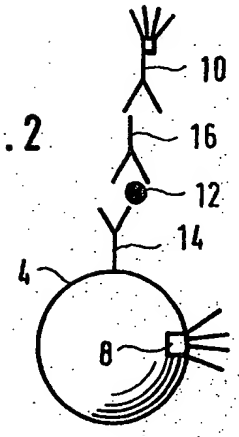


FIG.2



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